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(54) **NOVEL HYDROXAMIC ACID DERIVATIVE.**

(57) A novel peptide derivative compound which is expected to be useful for treatment of such diseases as rheumatoid arthritis, periodontal disease, corneal ulcer and epidermolysis bullosa, and which is a hydroxamic acid derivative of a tetrapeptide and has the action of specifically inhibiting collagenase of vertebrate origin.

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PRIOR ART

Some peptidylhydroxamic acids have heretofore been known as substances which exhibit inhibitory action on collagenase. Thus, William M. Moore et al. reported benzyloxycarbonyl-prolyl-leucyl-glycylhydroxamic acid (Z-Pro-Leu-Gly-NHOH) (see William M. Moore and Curtis A. Spilburg, Biochemical and Biophysical Research Communications, Vol. 136, No. 1, Pages 390-395, 1986). Furthermore as other peptide-based synthetic collagenase inhibitors were reported mercapto-containing compounds (see Robert D. Gray, Hossain H. Saneii and Arno F. Spatola, Biochemical and Biophysical Research Communications, Vol. 101, No. 4, Pages 1251-1258, 1981; Charles F. Vencill, David Rasnick, Katherine V. Crumley, Norikazu Nishino and James C. Powers, Biochemistry 24, 3149-3157, 1985) or carboxyl group-containing compounds (see Jean-Marie Delaisse, Yves Eeckhout, Christopher Sear, Alan Galloway, Keith McCullagh and Gilbert Vaes, Biochemical and Biophysical Research Communications, Vol. 133, No. 2, Pages 483-490, 1985).

The purpose of the present invention is to provide new peptide compounds which selectively inhibit the action of collagenase derived from vertebrates without inhibiting other protease actions (i.e. which exhibit an inhibitory action of high specificity), and which have low toxicity,

improved metabolic rate and other improved properties.

The present inventors, as a result of extensive researches aiming at developing new peptide compounds with such preferable properties have achieved the present invention, according to which it has been found that new peptidylhydroxamic acid derivatives of the general formula (I):

$$X^1-X^2-X^3-X^4-NHOH \quad (I)$$

wherein each of X^1, X^2, X^3 and X^4 is an α-amino acid residue; the carboxyl group of α-amino acid X^1 forms a peptide bond together with the amino group of α-amino acid X^2; the carboxyl group of α-amino acid X^2 forms a peptide bond together with the amino group of α-amino acid X^3; the carboxyl group of α-amino acid X^3 forms a peptide bond together with the amino group of α-amino acid X^4 and the carboxyl group of α-amino acid X^4 forms an amido bond together with -NHOH; and the hydrogen atom of the amino group in α-amino acid X^1 may be replaced by an aliphatic or aromatic carbyloxycarbonyl or acyl group which itself may have substituents, as well as their salts, are suitable for the purpose mentioned above.

The present inventors have succeeded in providing new compounds of the general formula (I) suitable for the purpose mentioned above by using, as an index, inhibitory action on each of the seven enzymes, i.e. collagenase from

human fibroblasts, collagenase from tadpoles, collagenase from bacteria, urease, thermolysin, α-chymotrypsin and trypsin to screen compounds for strong inhibitory action on the first two enzymes.

The preparation of new peptidylhydroxamic acid derivatives of the general formula (I) are carried out by processes which can be divided roughly into (A) and (B) below:

(A) Process where a compound of the formula Boc-X^4-NHOBzl is used as starting material; the peptide chain is extended on the Boc-N group side first to form the group X^3-X^4-, which is converted, via the group X^2-X^3-X^4- into the group X^1-X^2-X^3-X^4-; and finally the O-benzyl on the hydroxamic acid side is eliminated to give the desired compound; and

(B) Process where a compound of the formula Boc-X^4-OR^2 is used as starting material to synthesize the corresponding peptide derivative:

X^1-X^2-X^3-X^4-OR^2 (R^2: methyl or ethyl group)

which is then reacted with hydroxylamine to give the desired compound.

In the above mentioned processes, any means conventionally used in the peptide synthetic chemistry may be employed as specific means for condensing amino acids for formation of peptide chains; for protecting with

protecting groups the amino, imino, carboxyl and/or hydroxyl groups which may be present in their structure; and for eliminating such protecting groups. Such means is described in detail in the literature, for example, in Tanpaku-shitsu Kagaku (Protein chemistry) I, Amino-san (Amino acid)•Peputido (Peptide), ed. by Shiro Akabori, Takeo Kaneko and Kozo Narita, Kyoritsu Shuppan, 1969.

As means for carrying out the condensation mentioned above there may be mentioned a variety of methods, for example, dicyclohexylcarbodiimide (DCC) method, N,N'-dimethylaminopropylethylcarbodiimide (WSCD) method, mixed acid anhydride method, azide method, active ester method, oxidation reduction method and DCC-additive (e.g. 1-hydroxybenzotriazole, N-hydroxysuccinimide and N-hydroxy-5-norbornene-2,3-dicarboxyimide). Where the reaction is carried out using a solvent, there may be used as such solvent N,N-dimethylformamide (DMF), tetrahydrofuran (THF), methylene chloride, dioxane and ethyl acetate or mixtures thereof.

As examples of the protecting groups mentioned above, there may be mentioned benzyloxycarbonyl (Z), t-butyloxycarbonyl (Boc), benzoyl (Bz), acetyl, formyl, p-methoxybenzyloxycarbonyl and trifluoroacetyl for amino or imino group; methyl (OMe), ethyl (OEt), t-butyl, benzyl (OBzl) and p-nitrobenzyl for carboxyl group; and acetyl,

benzyl, benzyloxycarbonyl and t-butyl for hydroxyl group. In the foregoing description of compounds or groups, the parenthesized signs are abbreviations standing for such compounds or groups, and these abbreviations are also used as such in the present specification.

As means for eliminating the protecting groups mentioned above, there may be mentioned, for example, catalytic hydrogenation method and methods using trifluoroacetic acid, hydrogen fluoride, hydrogen bromide, hydrogen chloride, sodium hydroxide, potassium hydroxide, etc.

As pharmacologically acceptable salts of compounds of the general formula (I) according to the present invention, there may be mentioned N-addition salts such as hydrochloride, hydrobromide, sulfate, phosphate, formate, acetate, propionate, malonate, succinate, lactate, oxalate and tartarate, and where the amino group is protected, sodium salt, potassium salt, magnesium salt, calcium salt, aluminum salt, piperidine salt, morpholine salt, dimethylamine salt, diethylamine salt, etc.

The new hydroxamic acid derivatives according to the invention have a potent inhibitory action on collagenase derived from vertebrates. In addition these compounds, as well as their metabolites produced in the body, are presumed to have extremely high safety since the

components constituting their structure are naturally occurring amino acids of high safety or derivatives thereof.

The following examples are illustrative of the new compounds of the invention as well as of processes for their preparation.

Abbreviations used in the specification including the working examples to represent amino acids and their derivatives or groups present in the structure of these, reagents, etc. are in accordance with signs customarily used in the field of peptide synthetic chemistry (see IUPAC-IUB Commission on Biological Nomenclature), and have the following meanings:

Gly : Glycine	Ala : Alanine
Ile : Isoleucine	Leu : Leucine
Pro : Proline	Val : Valine
Sar : Sarcosine	Phe : Phenylalanine
Nle : Norleucine	Ser : Serine
Glu : Glutamic acid	Gln : Glutamine
Lys : Lysine	Arg : Arginine
Pgl : Phenylglycine	Hyp : Hydroxyproline
thioPro : Thioproline	Asp : Aspartic acid
Asn : Asparagine	Tyr : Thyrosine
Trp : Tryptophane	DCC : Dicyclohexyl-carbodiimide

HOBt : 1-Hydroxybenzotriazole

HOSu : N-Hydroxysuccinimide

Ac : Acetyl Boc : t-Butyloxycarbonyl

Z : Benzyloxycarbonyl Bz : Benzoyl

HPA : 2-(p-Hydroxyphenyl)propionyl

ABA : p-Aminobenzoyl PTH : o-Phthalyl

HBA : p-Hydroxybenzoyl Bzl : Benzyl ether

OBzl : Benzyl ester OEt : Ethyl ester

OMe : Methyl ester TEA : Triethylamine

THF : Tetrahydrofuran DMF : N,N-dimethyl-formamide

DMSO : Dimethylsulfoxide

TLC : Thin layer chromatography on silica gel

Amino acids referred to in the specification, where there can be optical isomers, are in L-form unless otherwise expressly indicated.

Example 1

t-Butyloxycarbonyl-glycyl-L-prolyl-L-leucyl-glycylhydroxamic acid (Boc-Gly-Pro-Leu-Gly-NHOH)

(A) Synthesis of Boc-Gly-NHOBzl

$HCl \cdot NH_2OBzl$ (11.2g; 70.2 mmol) was suspended in DMF (100 ml) and TEA (11.2 ml; 80.0 mmol) was added dropwise under ice-cooling. HOBt (7.43g; 55.0 mmol) and Boc-Gly-OH (8.76g; 50.0 mmol) were then added and the mixture was

cooled with a coolant at -20°C. DCC (14.5g; 70.2 mmol) dissolved in CH_2Cl_2 (70 ml) was added dropwise. After the dropwise addition, the reaction was allowed to proceed for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and then washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 300g; eluted with AcOEt : n-Hexane (= 1 : 1) mixed solvent) to give Boc-Gly-NHOBzl (13g; 93%) as a pale yellow oil.

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.75 and R_f② = 0.63.

(B) Synthesis of HCl•Gly-NHOBzl

4.5N HCl/AcOEt (30 ml) was added under ice cooling to Boc-Gly-NHOBzl (5.0g; 17.8 mmol) obtained in (A). The mixture was brought back to room temperature and the reaction was carried out for 1 hour. The solvent was distilled off under reduced pressure and the residue was solidified with Et_2O to give Hcl•Gly-NHOBzl (3.60g; 93%) as a hydroscopic colorless powder.

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 5 : 2 : 1, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.45 and R_f② = 0.44.

(C) Synthesis of Boc-Leu-Gly-NHOBzl

HCl•Gly-NHOBzl (7.15g; 33.0 mmol) obtained in (B) was dissolved in a mixed solvent of DMF (20 ml) and THF (80 ml) and TEA (4.9 ml; 35.0 mmol) was added dropwise under ice cooling. After the dropwise addition, HOBt (4.19g; 31.0 mmol) and Boc-Leu-OH (product from azeotropic dehydration of the monohydrate (7.48g; 30.0 mmol)) were added and the mixture was cooled with a coolant at -20°C. After DCC (8.05g; 39.0 mmol) dissolved in THF (20 ml) was added dropwise, the reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were removed by filtration and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was then distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 600g; eluted with AcOEt : n-hexane (= 2 : 1) mixed solvent) and then recrystallized from AcOEt-n-hexane mixed solvent to give Boc-Leu-Gly-NHOBzl (10.9g; 93%) as colorless needles.

m.p. 109 - 113°C, specific rotation $[\alpha]_D^{28}$ -8.3 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 80 : 10 : 5; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.58 and R_f② = 0.79.

(D) Synthesis of Boc-Gly-Pro-OEt

HCl•Pro-OEt (10.8g; 60.1 mmol) was dissolved in THF (70 ml). After TEA (8.4 ml; 60.0 mmol) was added dropwise under ice cooling, HOBt (7.43g; 55.0 mmol) and Boc-Gly-OH (8.76g; 50.0 mmol) were added. The mixture was cooled with a coolant at -20°C and DCC (13.4g; 65.0 mmol) dissolved in THF (30 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were removed by filtration and the solvent was distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 250g; eluted with AcOEt : n-hexane (= 3 : 2) mixed solvent) and then recrystallized from AcOEt-n-hexane to give Boc-Gly-Pro-OEt (10.5g; 85%) as colorless plates. m.p. 56.5 - 57.0°C, specific rotation $[\alpha]_D^{28}$ -84.9 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 80 : 10 : 5; color developing method

: 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.73 and R_f② = 0.90.

(E) Synthesis of Boc-Gly-Pro-OH

Boc-Gly-Pro-OEt (4.11g; 15.0 mmol) obtained in (D) was dissolved in MeOH (30 ml). The reaction was carried out for 1 hour after 2N-NaOH (10 ml) was added under ice cooling, and for 4 hours after the mixture was brought back to room temperature. The MeOH was distilled off under reduced pressure and the pH was adjusted to 2 with 1N-HCl. The mixture was extracted three times with AcOEt. The extracts were washed with water and dried over anhydrous $MgSO_4$, and the solvent was distilled off under reduced pressure. The residue was recrystallized from an AcOEt-n-hexane mixed solvent to give Boc-Gly-Pro-OH (3.44g; 93%) as colorless plates. m.p. 140.5 - 141°C , specific rotation $[\alpha]_D^{28}$ -75.5 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.51 and R_f② = 0.51.

(F) Synthesis of Boc-Gly-Pro-Leu-Gly-NHOBzl

To Boc-Leu-Gly-NHOBzl (3.93g; 10.0 mmol) obtained in (C) was added under ice cooling 4.5N-HCl/AcOEt (40 ml). The mixture was brought back to room temperature and the

reaction was then carried out for 1.5 hours. The solvent was distilled off under reduced pressure and the residue was dissolved in DMF (20 ml) and then cooled with a coolant at -20°C. After TEA (1.4 ml; 10 mmol) was added dropwise, HOBt (1.35g; 10.0 mmol) and Boc-Gly-Pro-OH (2.34g; 9.50 mmol) obtained in (E) were added and DCC (2.68g; 13.0 mmol) dissolved in THF (10 ml) was added dropwise. The reaction was carried out at -10°C for 1 hour and overnight in a refrigerator. After insolubles were removed by filtration, the solvent was distilled off under reduced pressure and the residue was dissolved in AcOEt and washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 250g; eluted with $CHCl_3$: MeOH (= 20 : 1) mixed solvent) to give Boc-Gly-Pro-Leu-Gly-NHOBzl (4.7g; 90%) as a colorless oil.

Specific rotation $[\alpha]_D^{28}$ -68.6 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 80 : 10 : 5; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.37 and R_f② = 0.72.

(G) Synthesis of Boc-Gly-Pro-Leu-Gly-NHOH

Boc-Gly-Pro-Leu-Gly-NHOBzl (1.0g; 1.83 mmol) obtained in (F) was dissolved in MeOH (20 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 1 hour using 10% Pd-C (50% wet) (0.3g). The catalyst was removed by filtration and the solvent was then distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 15g; eluted with $CHCl_3$: MeOH (= 20 : 1) mixed solvent) and then resolidified from $CHCl_3$-Et_2O mixed solvent to give Boc-Gly-Pro-Leu-Gly-NHOH (0.70g; 84%) as a colorless powder. m.p. 90 - 104°C, specific rotation $[\alpha]_D^{28}$ -84.3 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ - spraying) gave single spots at R_f① = 0.34 and R_f② = 0.67.

Example 2

t-Butyloxycarbonyl-glycyl-L-prolyl-L-leucyl-L-alanylhydroxamic acid (Boc-Gly-Pro-Leu-Ala-NHOH)

(A) Synthesis of Boc-Ala-NHOBzl

HCl•NHOBzl (2.07g; 13.0 mmol) was dissolved in a mixed solvent of DMSO (10 ml) and DMF (30 ml), and TEA (2.0 ml;

14.3 mmol) was added dropwise under ice cooling. After the dropwise addition, HOBt (1.35g; 10.0 mmol) and Boc-Ala-OH (1.89g; 10.0 mmol) were added and the mixture was cooled with a coolant at -20°C. DCC (2.70g; 13.1 mmol) dissolved in CH_2Cl_2 (10 ml) was added, and the reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were removed by filtration and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the residue was purified by chromatography on silica gel (Fuji Davison BW 200, 170g; eluted with AcOEt : n-hexane = 2 : 3 mixed solvent) and then recrystallized from an AcOEt-n-hexane mixed solvent to give Boc-Ala-NHOBzl (2.68g; 91%) as colorless needles. m.p. 98 - 99°C, specific rotation $[\alpha]_D^{28}$ -42.1 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 20 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.60 and R_f② = 0.58.

(B) Synthesis of Boc-Leu-Ala-NHOBzl

To Boc-Ala-NHOBzl (1.47g; 4.99 mmol) obtained in (A) was added under ice cooling 4.5N-HCl/AcOEt (10 ml). After

the mixture was brought back to room temperature, the reaction was carried out for 1 hour. The solvent was distilled off under reduced pressure and the residue was dissolved in THF (20 ml) and cooled with a coolant at -20°C. TEA (0.84 ml; 6.0 mmol) was added dropwise and HOBt (0.68g; 5.03 mmol) and Boc-Leu-OH (product from azeotropic dehydration with benzene of the monohydrate (1.17g; 4.69 mmol)) were added. DCC (1.35g; 6.50 mmol) dissolved in THF (5 ml) was added dropwise, and the reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were removed by filtration and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was distilled off under reduced pressure. The residue was solidified from AcOEt to give Boc-Leu-Ala-NHOBzl (1.53g; 80%) as colorless crystals. m.p. 164 - 166°C, specific rotation $[\alpha]_D^{28}$ -46.0 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 20 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.52 and R_f② = 0.60.

(C) Synthesis of Boc-Gly-Pro-Leu-Ala-NHOBzl

To Boc-Leu-Ala-NHOBzl (1.37g; 3.36 mmol) obtained in (B) was added under ice cooling 4.5N-HCl/AcOEt (10 ml). After the mixture was brought back to room temperature the reaction was carried out for 2 hours. The solvent was distilled off under reduced pressure and the residue was dissolved in DMF (10 ml) and cooled with a coolant at -20°C. After TEA (0.49 ml; 3.50 mmol) was added dropwise, HOBt (0.43g; 3.18 mmol) and Boc-Gly-Pro-OH (0.75g; 3.05 mmol) obtained in Example 1 (E) were added. DCC (0.83g; 4.00 mmol) dissolved in THF (5 ml) was added dropwise and the reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were removed by filtration and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 100g; eluted with $CHCl_3$: MeOH (= 20 : 1) mixed solvent) and then recrystallized from an AcOEt-n-hexane mixed solvent to give Boc-Gly-Pro-Leu-Ala-NHOBzl (1.32g; 81%) as colorless crystals. m.p. 103 - 105°C, specific rotation $[\alpha]_D^{28}$ -72.1 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ②

$CHCl_3$: MeOH : AcOH = 80 : 10 : 5; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.36 and R_f② = 0.80.

(D) Synthesis of Boc-Gly-Pro-Leu-Ala-NHOH

Boc-Gly-Pro-Leu-Ala-NHOBzl (0.53g; 0.94 mmol) obtained in (C) was dissolved in MeOH (10 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 1 hour using 10% Pd-C (Engelhard 50% wet) (0.17g). The catalyst was filtered off and the solvent was distilled off under reduced pressure. The residue was resolidified from a MeOH-Et_2O mixed solvent to give Boc-Gly-Pro-Leu-Ala-NHOH (0.40g; 86%) as a colorless powder. m.p. 112 - 118°C, specific rotation $[\alpha]_D^{28}$ -85.0 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ - spraying) gave single spots at R_f① = 0.39 and R_f② = 0.67.

Example 3

t-Butyloxycarbonyl-glycyl-L-prolyl-L-phenylalanyl-glycylhydroxamic acid (Boc-Gly-Pro-Phe-Gly-NHOH)

(A) Synthesis of Boc-Phe-Gly-NHOBzl

HCl·Gly-NHOBzl (2.38g; 11.0 mmol) obtained in Example

1 (B) was dissolved in a mixed solvent of DMF (6 ml) and THF (15 ml), and the solution was cooled with a coolant at -20°C. After TEA (1.54 ml; 11.0 mmol) was added dropwise, HOBt (1.42g; 10.5 mmol) and Boc-Phe-OH (2.65g; 10.0 mmol) were added and DCC (2.68g; 13.0 mmol) dissolved in CH_2Cl_2 (10 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was distilled off under reduced pressure. The residue was purified by chromatography on silica gel (Fuji Davison BW 200, 100g; eluted with $CHCl_3$: MeOH = 50 : 1 mixed solvent) and solidified from benzene to give Boc-Phe-Gly-NHOBzl (3.75g; 88%) as a colorless powder. m.p. 71 - 72°C, specific rotation $[\alpha]_D^{28}$ +9.8 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.75 and R_f② = 0.62.

(B) Synthesis of Boc-Gly-Pro-Phe-Gly-NHOBzl

To Boc-Phe-Gly-NHOBzl (2.75g; 6.44 mmol) obtained in

(A) was added under ice cooling 4.5N-HCl/AcOEt (20 ml). The mixture was brought back to room temperature and the reaction was carried out for 1 hour. Et_2O (30 ml) was added and insolubles precipitated thereby were then filtered off and dissolved in DMF (10 ml). The solution was cooled with a coolant at -20°C and TEA (0.90 ml; 6.44 mmol) was added dropwise. HOBt (0.83g; 6.14 mmol) and Boc-Gly-Pro-OH (1.59g; 5.85 mmol) obtained in Example 1 (E) were then added and DCC (1.57g; 7.61 mmol) dissolved in THF (5 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were removed by filtration and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively in water, 1N-HCl, water, 10% Na_2CO_3 and water. The solution was dried over anhydrous $MgSO_4$ and the solvent was distilled off under reduced pressure. The residue was solidified from a small volume of AcOEt to give Boc-Gly-Pro-Phe-Gly-NHOBzl (2.88g; 85%) as a colorless powder. m.p. 87 - 90°C, specific rotation $[\alpha]_D^{28}$ -71.8 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single

spots at R_f① = 0.66 and R_f② = 0.48.

(C) Synthesis of Boc-Gly-Pro-Phe-Gly-NHOH

Boc-Gly-Pro-Phe-Gly-NHOBzl (0.80g; 1.38 mmol) was dissolved in MeOH (10 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 2 hours using 10% Pd-C (Engelhard, 50% wet; 0.14g). The catalyst was removed by filtration and the solvent was distilled off under reduced pressure. The residue was solidified from a MeOH-Et_2O mixed solvent to give Boc-Gly-Pro-Phe-Gly-NHOH (0.46g; 68%) as a colorless powder. m.p. 166 - 171°C, specific rotation $[\alpha]_D^{28}$ -89.7 (c=1.0, MeOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ - spraying) gave single spots at R_f① = 0.44 and R_f② = 0.71.

Example 4

Benzoyl-glycyl-L-prolyl-L-leucyl-glycyl-hydroxamic acid (Bz-Gly-Pro-Leu-Gly-NHOH)

(A) Synthesis of Bz-Gly-Pro-Leu-Gly-NHOBzl

To Boc-Gly-Pro-Leu-Gly-NHOBzl (0.55g; 1.00 mmol) obtained in Example 1 (F) was added under ice cooling 4.5N HCl/AcOEt (2 ml), and the mixture was brought back to room

temperature. The reaction was carried out for 1 hour. The solvent was distilled off under reduced pressure and the residue was dissolved in DMF (5 ml). The solution was cooled with a coolant at -20°C and TEA (0.14 ml; 1.00 mmol) was added dropwise. Bz-Cl (0.17g; 1.21 mmol) was then added dropwise and TEA was used to adjust the pH to 8 - 9. The reaction was carried out for 1 hour and insolubles were filtered off. The solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt, and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and then dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was purified by chromatography on silica gel (Fuji Davison BW 200, 25g; eluted with $CHCl_3$: MeOH (=20:1) mixed solvent) to give Bz-Gly-Pro-Leu-Gly-NHOBzl (0.43g; 78%) as a colorless powder. m.p. 79 - 84°C, specific rotation $[\alpha]_D^{28}$ -69.0 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin - and then 47% hydrobromic acid - spraying followed by heating) gave single spots at R_f① = 0.22 and R_f② = 0.65.

(B) Synthesis of Bz-Gly-Pro-Leu-Gly-NHOH

Bz-Gly-Pro-Leu-Gly-NHOBzl (0.30g; 0.54 mmol) obtained

in (A) was dissolved in MeOH (10 ml) and the solution was subjected to catalytic hydrogenation for 3.5 hours at room temperature using 5% Pd-C (Engelhard, 50% wet; 0.10g). After the catalyst was filtered off, the solvent was distilled off under reduced pressure and the residue was recrystallized from an AcOEt-n-hexane mixed solvent to give Bz-Gly-Pro-Leu-Gly-NHOH (0.16g; 65%) as a colorless powder. m.p. 118 -123°C, specific rotation $[\alpha]_D^{28}$ -77.4 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ spraying) gave single spots at R_f① = 0.23 and R_f② = 0.60.

Example 5

t-Butyloxycarbonyl-glycyl-L-hydroxyprolyl-L-leucyl-glycylhydroxamic acid (Boc-Gly-Hyp-Leu-Gly-NHOH)

(A) Synthesis of Boc-Hyp-Leu-Gly-NHOBzl

To Boc-Leu-Gly-NHOBzl (3.89g; 9.89 mmol) obtained in Example 1 (C) was added under ice cooling 4.5N-HCl/AcOEt (30 ml), and the solution was brought back to room temperature. The reaction was carried out for 1 hour. The solvent was distilled off under reduced pressure and

the residue was dissolved in THF (100 ml). The solution was cooled with a coolant at -20°C. After TEA (1.40 ml; 10.0 mmol) was added dropwise, HOBt (1.22g; 9.03 mmol) and Boc-Hyp-OH (1.99g; 8.60 mmol) were added and DCC (2.31g; 11.2 mmol) dissolved in THF (10 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the solvent was then distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and then dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was recrystallized from AcOEt to give Boc-Hyp-Leu-Gly-NHOBzl (2.45g; 56%) as colorless crystals. m.p. 168 - 173°C, specific rotation $[\alpha]_D^{28}$ -50.8 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.40 and R_f② = 0.19.

(B) Synthesis of Boc-Gly-Hyp-Leu-Gly-NHOBzl

To Boc-Hyp-Leu-Gly-NHOBzl (2.00g; 3.95 mmol) obtained in (A) was added under ice cooling 4.5N-HCl/AcOEt (10 ml) and the mixture was brought back to room temperature. The

reaction was carried out for 1 hour. The precipitate was filtered off and dissolved in DMF (10 ml). TEA (0.55 ml; 3.95 mmol) was added dropwise under ice cooling, and Boc-Gly-ONSu (2.30g; 7.87 mmol) was added. The mixture was brought back to room temperature and the reaction was carried out for 3 hours. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and then dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was purified by chromatography on silica gel (Fuji Davison BW 200, 100g; eluted with $CHCl_3$: MeOH (=30:1) mixed solvent) to give Boc-Gly-Hyp-Leu-Gly-NHOBzl (1.57g; 70%) as a colorless oil. Specific rotation $[\alpha]_D^{28}$ -55.5 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.37 and R_f② = 0.64.

(C) Synthesis of Boc-Gly-Hyp-Leu-Gly-NHOH

Boc-Gly-Hyp-Leu-Gly-NHOBzl (0.75g; 1.33 mmol) obtained in (B) was dissolved in MeOH (10 ml) and the solution was subjected to catalytic hydrogenation for 1 hour at room temperature using 10% Pd-C (Engelhard, 50% wet; 0.25g).

The catalyst was filtered off and the solvent was distilled off under reduced pressure. The residue was resolidified from a MeOH-Et_2O mixed solvent to give Boc-Gly-Hyp-Leu-Gly-NHOH (0.5g; 79%) as a colorless powder. m.p. 178 - 183°C, specific rotation $[\alpha]_D^{28}$ -73.8 (c=1.0, MeOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 5 : 2 : 1, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin spraying followed by heating, (b) 10% Na_2CO_3- and then 5% $FeCl_3$- spraying) gave single spots at R_f① = 0.61 and R_f② = 0.51.

Example 6

p-Aminobenzyl-glycyl-L-prolyl-D-leucyl-D-alanyl-hydroxamic acid acetate

(AcOH•ABA-Gly-Pro-D-Leu-D-Ala-NHOH)

(A) Synthesis of Z-D-Leu-D-Ala-OMe

HCl•D-Ala-OMe (5.58g; 40.0 mmol) was dissolved in DMF (100 ml) and TEA (5.6 ml; 40.0 mmol) was added dropwise under ice cooling. After HOSu (2.30g; 20.0 mmol) and Z-D-Leu-OH (9.29g; 35.0 mmol) were added, the mixture was cooled with a coolant at -20°C and DCC (9.28g; 45.0 mmol) dissolved in CH_2Cl_2 (50 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the

solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was solidified from an Et_2O-n-hexane mixed solvent to give Z-D-Leu-D-Ala-OMe (11.5g; 94%) as a colorless powder. m.p. 94 - 95°C, specific rotation $[\alpha]_D^{28}$ +35.9 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin - and then 47% hydrobromic acid spraying followed by heating) gave single spots at R_f① = 0.82 and R_f② = 0.78.

(B) Synthesis of Boc-Pro-D-Leu-D-Ala-OMe

Z-D-Leu-D-Ala-OMe (8.80g; 25.1 mmol) obtained in (A) was dissolved in MeOH (80 ml) and 4.5N-HCl/AcOEt (10 ml) was added. The mixture was subjected to catalytic hydrogenation for 4 hours at room temperature using 10% Pd-C (Engelhard, 50% wet; 1.2g). The catalyst was filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in THF (50 ml) and the solution was cooled with a coolant at -20°C. After TEA (3.50 ml; 25.0 mmol) was added dropwise, HOSu (1.73g; 15.0 mmol) and Boc-Pro-OH (5.38g; 25.0 mmol) were

added and DCC (6.81g; 33.0 mmol) dissolved in CH_2Cl_2 (30 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was solidified from an Et_2O-n-hexane mixed solvent to give Boc-Pro-D-Leu-D-Ala-OMe (8.27g; 85%) as a colorless powder. m.p. 153 - 157°C, specific rotation $[\alpha]_D^{28}$ +11.6 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin spraying followed by heating) gave single spots at R_f① = 0.80 and R_f② = 0.63.

(C) Synthesis of Z-Gly-Pro-D-Leu-D-Ala-OMe

To Boc-Pro-D-Leu-D-Ala-OMe (4.13g; 10.0 mmol) obtained in (B) was added under ice cooling 4.5N-HCl/AcOEt (30 ml) and the mixture was brought back to room temperature and the reaction was carried out for 1.5 hours. The solvent was distilled off under reduced pressure and the residue was dissolved in DMF (30 ml). The solution was cooled with a coolant at -20°C. After TEA (1.40 ml; 10.0 mmol)

was added dropwise, HOSu (0.58g; 5.04 mmol) and Z-Gly-OH (2.10g; 10.0 mmol) were added and DCC (2.60g; 12.6 mmol) dissolved in CH_2Cl_2 (10 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was recrystallized from an AcOEt-Et_2O mixed solvent to give Z-Gly-Pro-D-Leu-D-Ala-OMe (3.95g; 78%) as colorless crystals. m.p. 130 - 134°C, specific rotation $[\alpha]_D^{28}$ +11.8 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin - and then 47% hydrobromic acid spraying followed by heating) gave single spots at R_f① = 0.74 and R_f② = 0.58.

(D) Synthesis of Z-ABA-Gly-Pro-D-Leu-D-Ala-OMe

Z-Gly-Pro-D-Leu-D-Ala-OMe (2.00g; 3.96 mmol) obtained in (C) was dissolved in MeOH (10 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 2 hours using 10% Pd-C (Engelhard, 50% wet; 0.50g). The catalyst was filtered off and the solvent was

distilled off under reduced pressure. The residue was dissolved in DMF (15 ml) and the solution was cooled with a coolant at -20°C. HOBt (0.27g; 2.00 mmol) and Z-ABA-OH (1.09g; 4.02 mmol) were added in that order, and DCC (1.03g; 4.99 mmol) dissolved in CH_2Cl_2 (5 ml) was added dropwise. The reaction was carried out for 1 hour at -10°C and overnight in a refrigerator. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was recrystallized from AcOEt to give Z-ABA-Gly-Pro-D-Leu-D-Ala-OMe (1.78g; 72%) as a colorless powder. m.p. 109 - 112°C, specific rotation $[\alpha]_D^{28}$ +4.7 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin - and then 47% hydrobromic acid - spraying followed by heating) gave single spots at R_f① = 0.65 and R_f② = 0.46.

(E) Synthesis of Z-ABA-Gly-Pro-D-Leu-D-Ala-NHOH

To Z-ABA-Gly-Pro-D-Leu-D-Ala-OMe (1.68g; 2.69 mmol) obtained in (D) was added under ice cooling a separately

prepared 1M NH_2OH/MeOH solution [i.e. a solution obtained by adding dropwise under ice cooling $NH_2OH \cdot HCl$ (0.63g; 9.06 mmol) dissolved in MeOH (4 ml) to a solution of KOH (1.00g; 85%; 15.1 mmol) in MeOH (3 ml) and filtering off the precipitated KCl] (6 ml) and the reaction was carried out for 4 hours. 3N-HCl was used to adjust the pH to 2 and the precipitate was filtered off to give Z-ABA-Gly-Pro-D-Leu-D-Ala-NHOH (1.68g; quantitative) as a colorless powder. m.p. 189 - 191°C, specific rotation $[\alpha]_D^{28}$ +10.4 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : (a) 0.1% ninhydrin - and then 47% hydrobromic acid spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ spraying) gave single spots at R_f① = 0.40 and R_f② = 0.14.

(F) Synthesis of AcOH•ABA-Gly-Pro-D-Leu-D-Ala-NHOH

Z-ABA-Gly-Pro-D-Leu-D-Ala-NHOH (1.40g; 2.24 mmol) obtained in (E) was dissolved in an AcOH : water (= 2 : 1) mixed solvent (10 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 2.5 hours using 10% Pd-C (Engelhard, 50% wet; 0.35g). The catalyst was distilled off and the solvent was distilled off under reduced pressure. The residue was recrystallized from

EtOH to give AcOH·ABA-Gly-Pro-D-Leu-D-Ala-NHOH (0.93g; 75%) as a colorless powder. m.p. 213 - 218°C, specific rotation $[\alpha]_D^{28}$ +23.4 (c=0.5, H_2O).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : H_2O = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ - spraying) gave single spots at R_f① = 0.25 and R_f② = 0.58.

Example 7

p-Hydroxybenzoyl-glycyl-L-prolyl-D-leucyl-D-alanylhydroxamic acid

(HBA-Gly-Pro-D-Leu-D-Ala-NHOH)

(A) Synthesis of Bzl-HBA-Gly-Pro-D-Leu-D-Ala-OMe

Z-Gly-Pro-D-Leu-D-Ala-OMe (1.73g; 3.43 mmol) obtained in Example 6 (C) was dissolved in MeOH (5 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 2 hours using 10% Pd-C (Engelhard, 50% wet; 0.30g). The catalyst was filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in DMF (10 ml) and cooled with a coolant at -20°C. Bzl-HBA-Cl [prepared by dissolving Bzl-HBA-OH (1.17g; 5.15 mmol) in $SOCl_2$ (5 ml), heating the solution under reflux for 3 hours and distilling off the

excess $SOCl_2$ under reduced pressure] dissolved in DMF (3 ml) was added dropwise. TEA was used to adjust the pH to 8 and the reaction was carried out for 4 hours. Insolubles were filtered off and the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt and the solution was washed successively with water, 1N-HCl, water, 10% Na_2CO_3 and water and dried over anhydrous $MgSO_4$. The solvent was distilled off under reduced pressure and the residue was subjected to chromatography on silica gel (Fuji Davison BW 200, 15g; eluted with AcOEt) to give Bzl-HBA-Gly-Pro-D-Leu-D-Ala-OMe (1.23g; 62%) as a colorless oil. Specific rotation $[\alpha]_D^{28}$ +4.6 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : 0.1% ninhydrin - and then 47% hydrobromic acid - spraying followed by heating) gave single spots at R_f① = 0.73 and R_f② = 0.58.

(B) Synthesis of Bzl-HBA-Gly-Pro-D-Leu-D-Ala-NHOH

To Bzl-HBA-Gly-Pro-D-Leu-D-Ala-OMe (1.15g; 1.98 mmol) obtained in (A) was added under ice cooling 1M-NH_2OH/MeOH (5 ml) prepared in the same manner as in Example 6 (E) and the reaction was carried out for 3 hours. 3N-HCl was used to adjust the pH to 2 and the precipitate was filtered off

and resolidified from a MeOH-Et_2O mixed solvent to give Bzl-HBA-Gly-Pro-D-Leu-D-Ala-NHOH (0.87g; 76%) as a colorless powder. m.p. 181 - 184°C, specific rotation $[\alpha]_D^{28}$ +13.6 (c=1.0, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH = 14 : 1, ② $CHCl_3$: MeOH : AcOH = 95 : 5 : 3; color developing method : (a) 0.1% ninhydrin - and then 47% hydrobromic acid - spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ - spraying) gave single spots at R_f① = 0.51 and R_f② = 0.25.

(C) Synthesis of HBA-Gly-Pro-D-Leu-D-Ala-NHOH

Bzl-HBA-Gly-Pro-D-Leu-D-Ala-NHOH (0.83g; 1.43 mmol) obtained in (B) was dissolved in MeOH (5 ml) and the solution was subjected to catalytic hydrogenation at room temperature for 2 hours using 10% Pd-C (Engelhard, 50% wet; 0.15g). The catalyst was filtered off and the solvent was distilled off under reduced pressure. The residue was solidified from a MeOH-Et_2O mixed solvent to give HBA-Gly-Pro-D-Leu-D-Ala-NHOH (0.53g; 76%) as a colorless powder. m.p. 159 - 164°C, specific rotation $[\alpha]_D^{28}$ +12.6 (c=0.5, EtOH).

TLC (developing solvent : ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5, ② n-BuOH : AcOH : water = 4 : 1 : 1; color developing method : (a) 0.1% ninhydrin - and then 47%

hydrobromic acid spraying followed by heating, (b) 10% Na_2CO_3 - and then 5% $FeCl_3$ - spraying) gave single spots at R_f① = 0.27 and R_f② = 0.67.

Examples 8 - 42

In accordance with the procedure as described in Examples 1 - 7, the compounds indicated in Table 1 were prepared. Data for the compounds obtained in the respective Examples are as shown in Table 1.

Table 1

Example No.	Compound (as indicated by formula)	m.p.°C	$[\alpha]_D^{28}$	TLC:R_f[1)]	Process for synthesis
8	HCl•Gly-Pro-Leu-Gly-NHOH	hygroscopic	-87.2(c=0.5, EtOH)	③0.24	A
9	HCl•Sar-Pro-Leu-Gly-NHOH	hygroscopic	-79.1(c=1.0, EtOH)	②0.19	A
10	Ac-Gly-Pro-Leu-Gly-NHOH	109 ~ 115	-91.3(c=1.0, EtOH)	①0.13②0.43	A
11	HCl • Bzl-Gly-Pro-Leu-Gly-NHOH	hygroscopic	-72.6(c=0.5, EtOH)	①0.09②0.45	A
12	Boc-Sar-Pro-Leu-Gly-NHOH	94 ~ 99	-80.6(c=1.0, EtOH)	①0.42②0.66	A
13	Ac-Sar-Pro-Leu-Gly-NHOH	hygroscopic	-88.2(c=0.5, EtOH)	①0.15②0.37	A
14	Boc\ Gly-Pro-Leu-Gly-NHOH Bzl/	hygroscopic	-73.8(c=0.5, EtOH)	①0.54②0.71	A
15	Boc-Gly-Pro-Leu-ß-Ala-NHOH	108 ~ 112	-70.2(c=1.0, EtOH)	①0.38②0.64	A
16	Boc-Gly-Pro-Leu-GAB-NHOH	88 ~ 93	-56.9(c=1.0, EtOH)	①0.38②0.64	A

Example No.	Compound (as indicated by formula)	m.p.°C	$[\alpha]_D^{28}$	TLC:R_f[1)]	Process for synthesis
17	Boc-Gly-Pro-Leu-D-Ala-NHOH	115 ~ 120	-89.3(c=1.0, EtOH)	①0.55②0.70	A
18	Boc-Gly-Pro-Leu-Val-NHOH	118 ~ 123	-113.6(c=1.0, EtOH)	①0.53②0.73	A
19	HCl·Gly-Pro-Leu-ß-Ala-NHOH	hygroscopic	-21.8(c=1.0, EtOH)	③0.27	A
20	HCl·Gly-Pro-Leu-GAB-NHOH	hygroscopic	-51.5(c=1.0, EtOH)	③0.22	A
21	Boc-Gly-Pro-D-Leu-Gly-NHOH	96 ~100	-20.3(c=1.0, EtOH)	①0.50②0.73	A
22	Boc-Gly-Pro-Gln-Gly-NHOH	hygroscopic	-67.1(c=1.0, EtOH)	①0.08②0.43	A
23	Boc-Gly-Pro-Glu-Gly-NHOH	94 ~100	-64.9(c=1.0, EtOH)	①0.12②0.49	A
24	Boc-Gly-Pro-Gly-Gly-NHOH	178 ~ 180	-52.3(c=1.0, DMF)	①0.16②0.44	A
25	Boc-Gly-Pro-Ile-Gly-NHOH	126 ~ 129	-78.3(c=1.0, EtOH)	①0.37②0.64	A
26	Boc-Gly-Pro-Ser-Gly-NHOH	101 ~ 105	-72.1(c=1.0, EtOH)	①0.12②0.45	A

Example No.	Compound (as indicated by formula)	m.p.°C	$[\alpha]_D^{28}$	TLC:R_f[1)]	Process for synthesis
27	Boc-Gly-Pro-Lys-Gly-NHOH	hygroscopic	-56.5(c=1.0, EtOH)	②0.25	A
28	Boc-Gly-Pro-Pro-Gly-NHOH HCl	hygroscopic	-98.1(c=1.0, EtOH)	①0.31②0.46	A
29	HCl•Gly-Pro-Arg-Gly-NHOH	hygroscopic	-65.2(c=1.0, H_2O)	④0.16	A
30	Boc-Gly-Gly-Leu-Gly-NHOH	126 ~ 129	-12.5(c=1.0, EtOH)	①0.18②0.65	A
31	Boc-Gly-Ala-Leu-Gly-NHOH	149 ~ 152	-42.2(c=1.0, EtOH)	①0.24②0.67	A
32	Bz-Gly-D-Pro-Leu-Gly-NHOH	99 ~ 105	+18.4(c=1.0, EtOH)	①0.55②0.70	A
33	Bz-Gly-thioPro-Leu-Gly-NHOH	166 ~ 169	-96.8(c=0.5, MeOH)	①0.30②0.67	A
34	Bz-Gly-Pro-Leu-Ala-NHOH	202 ~ 204	-71.7(c=1.0, DMF)	①0.78②0.75	A
35	Bz-Gly-Pro-D-Leu-D-Ala-NHOH	172 ~ 174	+10.8(c=1.0, DMF)	①0.63②0.70	A
36	Bz-Gly-Pro-Leu-Sar-NHOH	107 ~ 110	-94.5(c=1.0, EtOH)	①0.44②0.58	A

Example No.	Compound (as indicated by formula)	m.p.°C	$[\alpha]_D^{28}$	TLC:R_f 1)	Process for synthesis
37	Bz-Gly-Pro-D-L-Sar-NHOH	116 ~ 119	-36.9(c=1.0, DMF)	①0.49②0.60	A
38	Bz-Gly-Pro-Leu-Leu-NHOH	131 ~ 135	-74.0(c=0.5, EtOH)	①0.35②0.66	A
39	Bz-Gly-Pro-D-Leu-D-Leu-NHOH	187 ~ 190	+25.6(c=0.5, EtOH)	①0.69②0.74	A
40	PTH-Gly-Pro-D-Leu-D-Leu-NHOH	159 ~ 164	+17.8(c=0.5, EtOH)	①0.15②0.51	A
41	AcOH·ABA-Gly-Pro-Leu-Ala-NHOH	229 ~ 232	-116(c=1.0, $^{AcOH:H_2O}_{=3:2}$)	①0.08②0.45	B
42	Bz-Gly-Pro-Leu-D-Ala-NHOH	119 ~ 123	-98.1(c=1.0, DMF)	①0.49②0.66	A

1) developing solvent: ① $CHCl_3$: MeOH : AcOH = 80 : 10 : 5
② n-BuOH : AcOH : H_2O = 4 : 1 : 1
③ n-BuOH : AcOH : H_2O = 4 : 2 : 1
④ n-BuOH : AcOH : H_2O = 4 : 3 : 3

Color developing method:
(a) 0.1% ninhydrin - and then 47% hydrobromic acid spraying followed by heating.
(b) 10% Na_2CO_3 - and then 5% $FeCl_3$-spraying

Using the following procedures, the new peptide compounds of the invention were assayed for inhibitory activity against collagenases as well as against other enzymes:

(1) Inhibitory activity against collagenases

The inhibitory activity against human fibroblast collagenase (collagenase derived from human fibroblasts), tadpole-derived collagenase and bacteria (Clostridium)-derived collagenase was assayed in accordance with the method of Nagai [see Ensho, 4(2), 123 (1984)] using a fluorescence-labeled collagen (FITC-derivatized bovine type I collagen).

(2) Inhibitory activity against urease

The inhibitory activity against urease was assayed in accordance with the method of Kobashi et al. [see Biochem. Biophys. Acta, 227 429 (1971)] using sword bean-derived urease.

(3) Inhibitory activity against thermolysin, trypsin and α-chymotrypsin

This was assayed in accordance with the method of Laskowski [see Meth. Enzymol., 2, 8 (1955)] using a thermally denatured casein as substrate for the respective enzymes (i.e. thermolysin, trypsin and α-chymotrypsin).

Results of these assays are shown in Table 2.

Table 2

Exmaple No.	IC_{50} (μM)		Inhibition (%)				
	Human fibroblast collage-nase	Tadpole collage-nase	Bacterial collage-nase	Urease	Thermolysin	Trypsin	α-Chymo-trypsin
6	1.28	1.10	7.1%/ 2.0×10^{-4}M	2.0%/ 4.0×10^{-3}M	26.8%/ 4.0×10^{-3}M	6.5%/ 2.0×10^{-3}M	26.4%/ 2.0×10^{-3}M
7	1.18	1.05	20.9%/ 4.0×10^{-3}M	5.3%/ 4.0×10^{-3}M	60.4%/ 4.0×10^{-3}M	56.1%/ 4.0×10^{-3}M	22.3%/ 4.0×10^{-3}M
18	3.1	9.8	50.0%/ 3.4×10^{-2}M	22.0%/ 2.0×10^{-2}M	50.0%/ 1.01×10^{-2}M	0%/ 4.0×10^{-3}M	0%/ 4.0×10^{-3}M
34	2.7	7.7	34.0%/ 1.2×10^{-3}M	0%/ 4.0×10^{-3}M	35.0%/ 4.0×10^{-3}M	0%/ 2.0×10^{-4}M	0%/ 2.0×10^{-4}M
35	6.4	3.1	33.0%/ 3.0×10^{-3}M	24.0%/ 3.0×10^{-3}M	38.0%/ 4.0×10^{-3}M	2.2%/ 1.0×10^{-4}M	12.8%/ 1.0×10^{-4}M
40	3.6	3.1	20.7%/ 4.0×10^{-3}M	3.3%/ 4.0×10^{-3}M	32.2%/ 4.0×10^{-3}M	21.6%/ 1.0×10^{-2}M	22.3%/ 1.0×10^{-2}M
41	3.7	4.0	0%/ 5.0×10^{-5}M	3.3%/ 3.4×10^{-5}M	4.0%/ 2.0×10^{-5}M	14.4%/ 2.0×10^{-5}M	27.3%/ 2.0×10^{-5}M

The new peptides of the invention are extremely useful since they are found to have a specific inhibitory activity against collagenase as compared to known peptide substances.

The toxicity of the new peptide compounds of this invention is as follows:

Acute toxicity test (LD_{50}) in mice

Compound (as indicated by Example No.)	LD_{50}	
	Intraperitoneal administration	Intravenous administration
6	>2g/kg	>200mg/kg
7	>2g/kg	>200mg/kg
41	>2g/kg	>200mg/kg

CLAIMS

1. Peptidylhydroxamic acid derivatives of the general formula:

$X^1 - X^2 - X^3 - X^4 - NHOH$ (I)

wherein each of X^1, X^2, X^3 and X^4 is an α-amino acid residue; the carboxyl group of α-amino acid X^1 forms a peptide bond together with the amino group of α-amino acid X^2; the carboxyl group of α-amino acid X^2 forms a peptide bond together with the amino group of α-amino acid X^3; the carboxyl group of α-amino acid X^3 forms a peptide bond together with the amino group of α-amino acid X^4 and the carboxyl group of α-amino acid X^4 forms an amido bond together with -NHOH; and the hydrogen atom of the amino group in α-amino acids X^1 may be replaced by an aliphatic or aromatic carbyloxycarbonyl or acyl group which itself may have substituents, or salts thereof.

2. Peptidylhydroxamic acid derivatives or salts thereof as claimed in claim 1 wherein X^1 in the formula (I) is a residue of an α-amino acid selected from glycine, alanine and sarcosine.

3. Peptidylhydroxamic acid derivatives or salts thereof as claimed in claim 1, wherein X^2 in the formula (I) is a residue of an amino acid selected from proline, hydroxyproline, thioproline and alanine.

4. Peptidylhydroxamic acid derivatives or salts as claimed in claim 1, wherein X^3 in the formula (I) is a residue of an amino acid selected from asparagine, glutamine, aspartic acid, glutamic acid, alanine, valine, leucine, isoleucine, norleucine, phenylglycine, phenylalanine, thyrosine and tryptophane.

5. Peptidylhydroxamic acid derivatives or salts thereof as claimed in claim 1, wherein X^4 in the formula (I) is a residue of an α-amino acid selected from glycine, alanine, valine, leucine, isoleucine, norleucine and sarcosine.

INTERNATIONAL SEARCH REPORT

0 345 359

International Application No PCT/JP88/01281

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl[4] C07K5/10, A61K37/64, C12N9/99

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07K5/00, A61K37/64, C12N9/99

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Eur. J. Med. Chem. - Chim. Ther., Vol. 18, No.6, (1983) Severo Salvadori, et al [Synthesis and Pharmacological Activity of Dermorphin Tetrapeptide-Analogs] pp. 489-493	1-5
X	Arzneim.-Forsch., Vol.33, No. 11, (1983) G. P. Sarto, et al [Pharmacological Studies of a Series of Dermorphin Related Tetrapeptides] pp. 1577-1579	1-5
X, Y	US, A, 4,687,841 (Monsanto Co.) 18 August 1987 (18. 08. 87) Columns 2 to 4 & US, A, 4720486	1-5
Y	JP, A, 62-103052 (G. D. Searle & Co.) 13 May 1987 (13. 05. 87) Pages 1 to 2 & US, A, 4599361	1-5
Y	JP, A, 61-152650 (G. D. Searle & Co.) 11 July 1986 (11. 07. 86) Pages 1 to 3 & US, A, 4595700	1-5

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
March 1, 1989 (01. 03. 89)	March 20, 1989 (20. 03. 89)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

International Application No. PCT/JP88/01281

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	& EP, A, 185380 & US, A, 4681966	
Y	JP, A, 61-103896 (G. D. Searle & Co.) 22 May 1986 (22. 05. 86) Pages 1 to 2 & EP, A, 159396 & US, A, 4568666	1-5
Y	EP, A, 214,639 (G. D. Searle & Co.) 18 March 1987 (18. 03. 87) Pages 3 to 5 & US, A, 4743587 & AU, A, 8662408	1-5

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.